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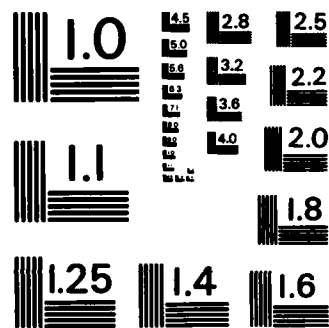
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		GOVT ACCESSION NO.		3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) INDIRECT IMMUNOFLOUORESCENCE, SERUM NEUTRALIZATION, AND VIREMIA RESPONSES OF RHESUS MONKEYS (MACACA MULATTA) TO MACHUPO VIRUS				5. TYPE OF REPORT & PERIOD COVERED	
				6. PERFORMING ORG. REPORT NUMBER	
7. AUTHOR(s) E. Gonder and G. Eddy, Dept. of Food Anim. & Equine Med., Sch. of Vet. Med., NC State Univ., Raleigh, NC (E.G.), and BioDx Assoc., 7005 Summer- field Dr., Frederick, MD (G.E.)				8. CONTRACT OR GRANT NUMBER(s)	
9. PERFORMING ORGANIZATION NAME AND ADDRESS SGRD-UIV, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD 21701-5011				10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	
11. CONTROLLING OFFICE NAME AND ADDRESS				12. REPORT DATE 22 Aug 85	
				13. NUMBER OF PAGES 16	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)				15. SECURITY CLASS. (of this report)	
				15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release: Distribution unlimited.					
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)					
18. SUPPLEMENTARY NOTES To be published in the Journal of Medical Virology.					
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) arenavirus, Machupo, Bolivian hemorrhagic fever, immunofluorescence, rhesus					
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Although indirect immunofluorescent antibody tests (IFAT) have been developed for several arenaviruses, none has been applied to the rhesus monkey model for Bolivian hemorrhagic fever (caused by the arenavirus Machupo). We infected eight rhesus monkeys with Machupo virus and bled them weekly postinoculation (PI) for determination of viremia and for serum antibody by IFAT and serum neutralization (SN) testing. Viremia peaked 14 days PI, when two of eight animals had low IFAT titers. At 21 days PI, the six surviving monkeys had					

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**INDIRECT IMMUNOFLUORESCENCE, SERUM NEUTRALIZATION, AND VIREMIA
RESPONSES OF RHESUS MONKEYS (MACACA MULATTA) TO MACHUPO VIRUS**

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SHORT TITLE: IFAT Response of Machupo-Infected Rhesus

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

All work was conducted at the United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011.

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ABSTRACT

Although indirect immunofluorescent antibody tests (IFAT) have been developed for several arenaviruses, none has been applied to the rhesus monkey model for Bolivian hemorrhagic fever (caused by the arenavirus Machupo). We infected eight rhesus monkeys with Machupo virus and bled them weekly postinoculation (PI) for determination of viremia and for serum antibody by IFAT and serum neutralization (SN) testing. Viremia peaked 14 days PI, when two of eight animals had low IFAT titers. At 21 days PI, the six surviving monkeys had elevated IFAT titers and diminished viremias. SN titers were not observed until 28 days PI, when three of four survivors had low titers. Results of the IFAT were available more rapidly than the SN, and detected increased serum antibody titers earlier than the SN.

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KEY WORDS

arenavirus, Machupo, Bolivian hemorrhagic fever, immunofluorescence,
rhesus

INTRODUCTION

The arenavirus Machupo is the etiologic agent of Bolivian hemorrhagic fever (BHF), described as a geographically delineated hemorrhagic fever with a human mortality rate varying from 5-30% in different outbreaks, with uncommon subclinical infections, and with infrequent person-to-person transmission [Johnson, 1967]. Because of the potential lethality of BHF for humans, a suitable laboratory model has been developed to evaluate potential therapeutic regimens. [Eddy, 1975; Kastello, 1976; Peters, 1984; and Stephen, 1980]. The serological technique described in the model was a plaque reduction, serum dilution, neutralizing antibody test that required 7 days to perform on confluent cell culture monolayers [Eddy, 1975; Webb, 1969]. Indirect fluorescent antibody testing (IFAT) has become a common serological tool for the evaluation of natural and laboratory arenavirus infection, commonly requiring only 2-4 hours to perform., and often provides an alternate spectrum of virus-specific antibody assessment to virus neutralization [Lewis, 1975; Peters, 1973, 1984; Wulff, 1975, 1978]. This report presents IFAT results from eight Machupo-infected monkeys, and results of a safety test conducted on antigen slides that conflicts with a previous report [Peters, 1973].

MATERIALS AND METHODS

Monkeys

Two groups of four rhesus monkeys were evaluated. Group 1 consisted of 5- to 8-kg monkeys [Eddy, 1975], and in Group 2 were 5- to 7-kg monkeys [Stephen, 1980; Jones, unpublished data]. Animals in both groups were healthy, untreated, virus-inoculated controls from other experimental studies with BHF, with no known exposure to any arenavirus prior to Machupo virus inoculation. Monkeys were caged singly in a biological containment facility [Kuehne, 1983], and observed daily for clinical signs. Blood samples for serology and viremia assays were obtained 7, 14, 21, and 28 days PI.

Virus inoculation and viral assay

All monkeys were inoculated subcutaneously with the Carvalho strain of Machupo virus [Johnson, 1964]. Group 1 received approximately 1,000 plaque-forming units (PFU) of the third suckling hamster brain passage; Group 2 received 32,000 PFU of the fourth passage. Assays for virus were performed as previously described [Kastello, 1976].

Serology

The serum neutralization test was a plaque reduction, serum dilution, neutralizing antibody test (SN) performed in Vero cells [Eddy, 1975]. The IFAT reagents and procedures used were similar to those described by Peters [1973], except that Vero cell monolayers were infected at a multiplicity of infection of 0.3-1.0 with fourth-passage Carvalho strain BHF virus. Cells adsorbed virus for 3-4 hours and were harvested three days PI. the infected monolayer from a single 25-cm² flask was trypsinized, suspended in 1.5 ml of Puck's saline A, and gently disrupted

by pipet. To avoid handling infected cells, there was no further attempt to standardize cell concentrations. The suspension (0.025 ml) was placed on each of seven spots on Teflon-coated microscope slides having eight, 8-mm circular, uncoated spots (Cell-Line Associates, Inc., Minotola, New Jersey) and allowed to air dry under ultraviolet irradiation. The remaining spot received a similar preparation of uninfected cells. Slides were then fixed in acetone at room temperature for 20 minutes and stored in air at -70°C until used.

Each serum dilution was applied to two spots on each slide, but no serum was applied to one infected spot or the uninfected spot. Following incubation, rinsing, and drying, FITC-conjugated, goat, anti-human immunoglobulin was applied to all spots receiving serum dilutions, and a FITC-conjugated monkey anti-Machupo antisera was applied to the remaining infected and uninfected cell spots as a direct fluorescent antibody test (DFAT) to control slide quality. Coverslips were mounted with glycerine and slides examined with a Zeiss microscope equipped for FITC epiluminescence. The last dilution of serum in which both infected cell spots showed brilliant apple-green granular cytoplasmic fluorescence similar to the DFAT-stained infected spot was considered the endpoint.

Safety test

By using a common inoculum, two different lots of antigen slides were prepared as described above and exposed to 15 min of unrated ultraviolet radiation in a passbox [Kuehne, 1973]. Cells from 10 infected spots from each lot were removed by placing a small amount of titration diluent on each spot and vigorously scraping with a small spatula until no cells remained after microscopic examination. The material from 10 spots was

diluted to 2.0 ml as a stock solution. The virus titer of this material was determined as described above; additionally, 0.2 ml of serial dilutions of this stock was inoculated into each of two Vero cell monolayers in 25-cm² tissue culture flasks. One of these flasks was harvested and antigen spot slides examined by DFAT three days PI as described above; the supernate from the other was sampled for virus titration at five days PI as described above.

RESULTS

Results of animal inoculation are summarized in Figure 1. Maximum viremia levels occurred on day 14 PI and declined thereafter; IFAT titers were detectable in two of eight monkeys on day 14 PI, and in all six monkeys surviving on 21 days PI. By 21 days PI, the sera from the six surviving monkeys had an IFAT geometric mean titer (GMT) of 3.1 (log₁₀). Neutralizing antibody developed more slowly, and did not appear until day 28 PI, when the four surviving monkeys exhibited a SN GMT of 0.68 (log₁₀). The mortality rate of the model, clinical condition of survivors after day 21 PI, and other experimental demands for convalescent antisera precluded additional testing. However, a previous report [Eddy, quoted in Peters, 1984] described SN titers in the Machupo-monkey model as increasing at a similar time PI.

The safety test results are summarized in Table I. Although virus activity was greatly reduced by acetone fixation followed by unrated ultraviolet exposure, as evidenced by inability to detect virus by immediate plaque titration of scrapings from antigen slides, slight activity remained and was detected by passage in cell culture. Results of

DFAT testing corresponded exactly to those of conventional plaque titration, but were available seven to eight days faster.

DISCUSSION

Others [de Bracco, 1978; Lewis, 1975; Peters, 1973; Wulff, 1975] have noted that IFAT results are useful for diagnosis of arenavirus infections, but have not emphasized the gains in diagnostic time possible relative to infection. Although truncated, our results confirm the ability of the IFAT, compared to the SN, to rapidly detect Machupo virus infection in rhesus monkeys, and of the DFAT, compared to plaque titration, to detect infection in cell culture. Fluorescent antibody tests can be performed in less than one day, compared to the minimum of seven days required to perform a SN and six days for a plaque titration. In the case of the described model, a presumptive diagnosis was obtained on day 14 and was confirmed by additional serology on day 21 PI by IFAT. In comparison, detectable SN titers were not evident until day 28 PI, and an additional seven days were required to perform the test; therefore, SN diagnosis was not available until day 34 PI, 13 days after a diagnosis could be established by IFAT. This interval could be very valuable in institution of therapy and control measures.

The principal disadvantage of the IFAT is that it is less specific for individual arenaviruses than the SN procedure. Substantial cross-reactivity by IFAT has been described between New World arenaviruses [Wulff, 1978], and a specific diagnosis to virus type required SN serology [Jahrling, 1980].

Machupo antigen on slides is largely inactivated by acetone fixation and ultraviolet exposure, but obviously should be used with caution. The

buffered formaldehyde fixation/trypsin digestion technique described by van der Groen [1983] may result in more complete inactivation so that prepared slides can be stored and shipped as described by Rosato [1982].

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Lauren (Pete) R. Bagley and Mr. Bill G. Mahlandt, whose technical expertise and attention to detail have proven worthy of emulation.

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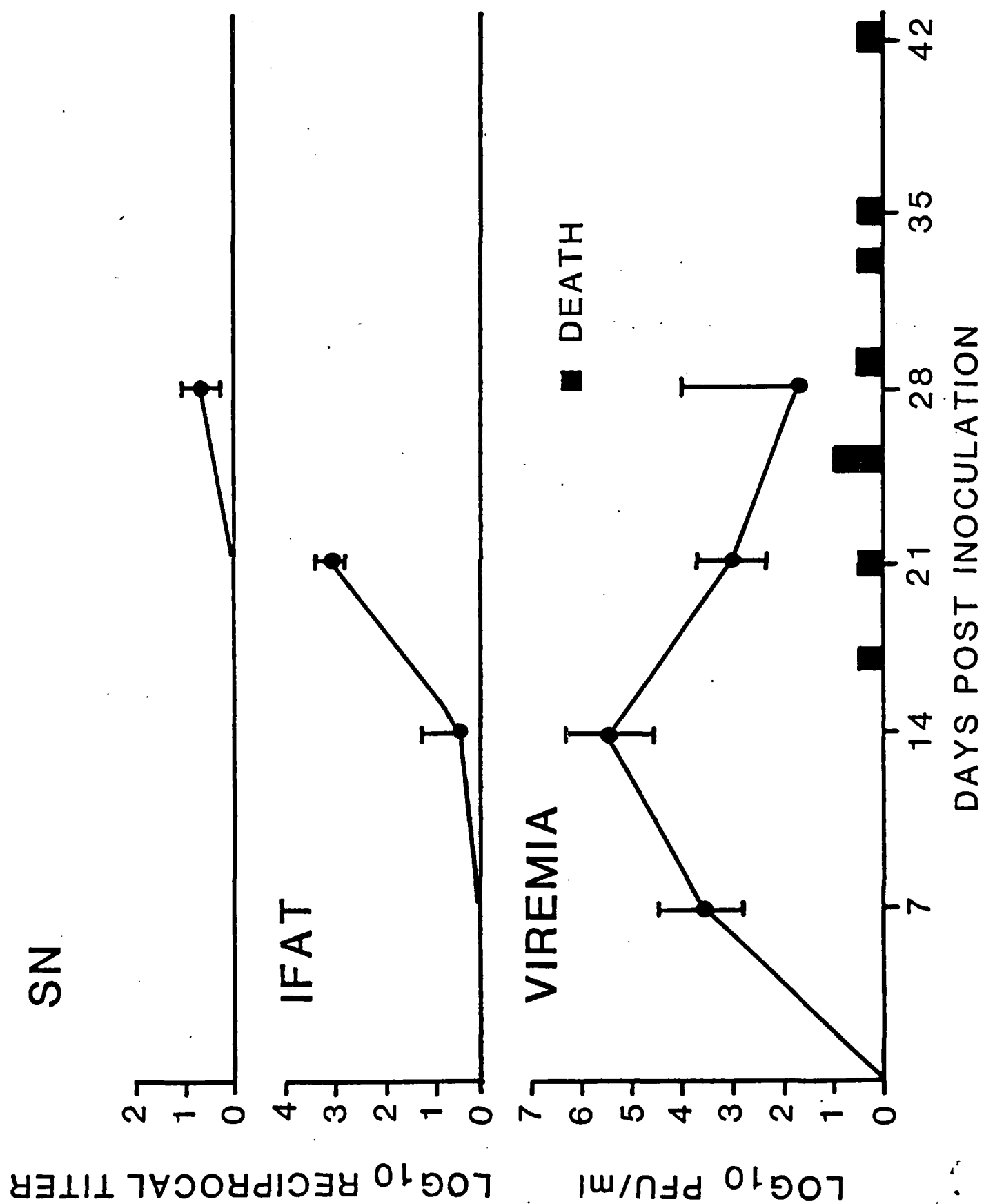
FIGURE LEGEND

Fig. 1. Serum neutralization (SN), indirect fluorescent antibody (IFAT), and viremia responses of rhesus monkeys after inoculation with Machupo virus. Viremia response is expressed as plaque-forming units (PFU)/ml.

Table I. Results of safety testing of acetone fixed/ultraviolet
exposed Machupo antigen spot slides

	<u>Lot #1</u>	<u>Lot #2</u>				
Plaque forming units (PFU)/ spot delivered prior to inactivation (\log_{10})	ND*	3.46				
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	Dilution of Stock (\log_{10})					
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PFU/spot after inactivation	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
% Cells DFAT-positive 3 days PI in flasks inoculated with stock dilutions	>30	>30	>30	>30	>30	>30
PFU/ml 5 days PI in flasks inoculated with stock dilutions	6.08	6.04	5.76	6.02	6.02	5.42

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